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Bacterial symbioses. Predation and mutually beneficial associations

Summary The endosymbiotic theory, which has proved to explain the origin of mitochondria and chloroplasts, also posits the origin of nucleus and other cellular organelles that could have derived from ancient relationships among bacteria. It seems that predation might have been a prerequisite to the establishment of symbiosis as a source of evolutionary novelty. This review describes current different examples of bacteria able not only to attack and degrade other bacteria, but also to establish stable symbiotic relationships with different eukaryotic organisms.

Key words Bacterial endosymbiosis · Microbial predation · Bacteria-protists relationships · Bacteria-plants relationships · Bacteria-animals relationships

Introduction

Several theories on the origin of eukaryotic cells have been proposed. One of them, the endosymbiotic theory, proposed by Margulis [46], claims that independent, free-living microbes joined together, first casually, then in more stable associations. As time passed and evolutionary pressures favored such symbiotic unions, the partner microbes became permanently joined in a new cell consisting of interdependent components. According to this theory, three classes of organelles (mitochondria, plastids, and undulipodia) once lived as independent prokaryotes [8, 21, 37].

At least two classes of eukaryotic organelles, used for respiration and for motility, have been suggested to have a directly detectable legacy from such prokaryotic predation. Extant intracellular structures, such as mitochondria, hydrogenosomes, kinetosomes, and axonemes of undulipodia are derived from ancient biotic relations among bacteria which resisted the stringent selection pressures of death by predation [3, 24, 32, 42].

The endosymbiotic theory also posits that the nucleus, like the other eukaryotic organelles enclosed in double membranes, was derived through capture by an engulfing species. The origin of the eukaryotic nucleus has been interpreted by Gupta [38] as an endosymbiotic event between two completely different prokaryotes. One of these, the host, he thinks arose from within the Gram-negative bacteria and the other, the guest, he thinks is most likely an eocyte (a group of hyperthermophilic sulfur metabolizing prokaryotes). The author bases his interpretations on the sequences of the 70 kDa heat shock protein (HSP70) [38].

Besides, it has also been suggested that the first eukaryote should have been a consequence of the symbiotic association between an anaerobic, strictly hydrogen-dependent, strictly autotrophic archaeobacterium (the host), and a eubacterium (the symbiont) that was able to respire, but generated molecular hydrogen as a waste product of the anaerobic heterotrophic metabolism [47]. Among the eukaryotes, trichomonads are the earliest to diverge from the main line of eukaryotic descent. In accordance with their ancient nature, these facultative anaerobic protists lack two organelles found in most eukaryotes: mitochondria and peroxisomes. Trichomonads do contain, however, an unusual organelle involved in carbohydrate metabolism, called the hydrogenosome. Hydrogenosomes lack DNA, cytochromes and citric acid cycle enzymes. Instead, they contain enzymes typically found in anaerobic bacteria, and are capable of producing molecular hydrogen [48].

Archaeozoan protists are thought to represent lineages that diverged from other eukaryotes before the acquisition of mitochondria and other organelles [9]. The parasite *Entamoeba histolytica* was originally included in this group. Ribosomal RNA-based phylogenies, however, place *E. histolytica* on a comparative recent branch of the eukaryotic tree, implying that its ancestors had these structures. Clark and Roger [11] showed direct evidence for secondary loss of mitochondrial function by isolating two *E. histolytica* genes which encode proteins that in other eukaryotes are found in the mitochondrion: the enzyme pyridine nucleotide transhydrogenase and one chaperonin.

Germot et al. [29] found something similar in *Trichomonas vaginalis*. This protist exhibits a fragment of sequence signature, so far found only in mitochondrial HSP70 and in proteobacterial DnaK. Thus, mitochondrial endosymbiosis might have occurred

earlier than previously assumed. The trichomonad double membrane-bound organelles, the hydrogenosomes, might have evolved from mitochondria.

Biotic selection pressures, which are fundamentally different from the abiotic ones in that they require organismic interaction, can also be recognized. Predation must have been a prerequisite to the theory that symbiosis is a source of evolutionary novelty. Some of the first partnerships between microorganisms must have been partially aggressive at first, and probably became stable with time. Nowadays, relationships of that kind can be frequently observed. We will mention a few examples of both predation and stable partnerships in which bacteria are the main characters.

Extracellular predation

Several predator-prey relations described here were studied in microorganisms living in karstic lakes [20, 25, 35]. In most cases, bacterial cell lysis and digestion require contact between the bacterium and its prey (intracellular and extracellular predation), but in a few cases they are caused by extracellular lytic enzymes [4, 16, 58]. Recently the lytic enzymes produced by *Stenotrophomonas* sp. against *Chlorobium* cells have been described [49]. As examples of extracellular predation, the following bacteria are described: *Ensifer*, *Micavibrio*, *Vampirovibrio* and *Vampirococcus*.

Ensifer adherens is an aerobic Gram-negative bacterium consisting of rods (0.7–1.1 x 1.0–1.9 µm) occurring singly or in pairs. It can attach to various living Gram-positive and Gram-negative bacteria but is not an obligate predator [5, 6].

Micavibrio admirandus is a Gram-negative curved and small (0.25–0.4 x 0.6–1.0 µm) bacterium, with a single polar unsheathed flagellum 15 nm in diameter. It attaches to the surface of the prey cells and destroys them without penetration [43].

Vampirovibrio chlorellavorus was described in 1972 by Gromov and Mankaeva [31]. This bacterium has an eukaryote (the protist *Chlorella*) as its only prey. Although it exhibits certain similarities with *Bdellovibrio*, it differs from it in important traits: elongated spirillar forms do not occur; growth occurs outside the prey cell, which is not penetrated, and finally the flagellum lacks a sheath.

Vampirococcus was first described in 1983 by Esteve et al. [23] in the course of ecological studies on the phototrophic bacterial communities of sulfurous karstic lakes. *Vampirococcus* is a Gram-negative, ovoidal (0.6 µm wide) bacterium, which does not have any flagellum, is apparently an obligate anaerobe, and seems to multiply only when attached to its prey (Fig. 1A).

Intracellular predation

Isolated by Stolp and first described by Stolp and Petzold in 1962, *Bdellovibrio* [57] has been the most well characterized bacterium with predatory activity against other bacteria [15,

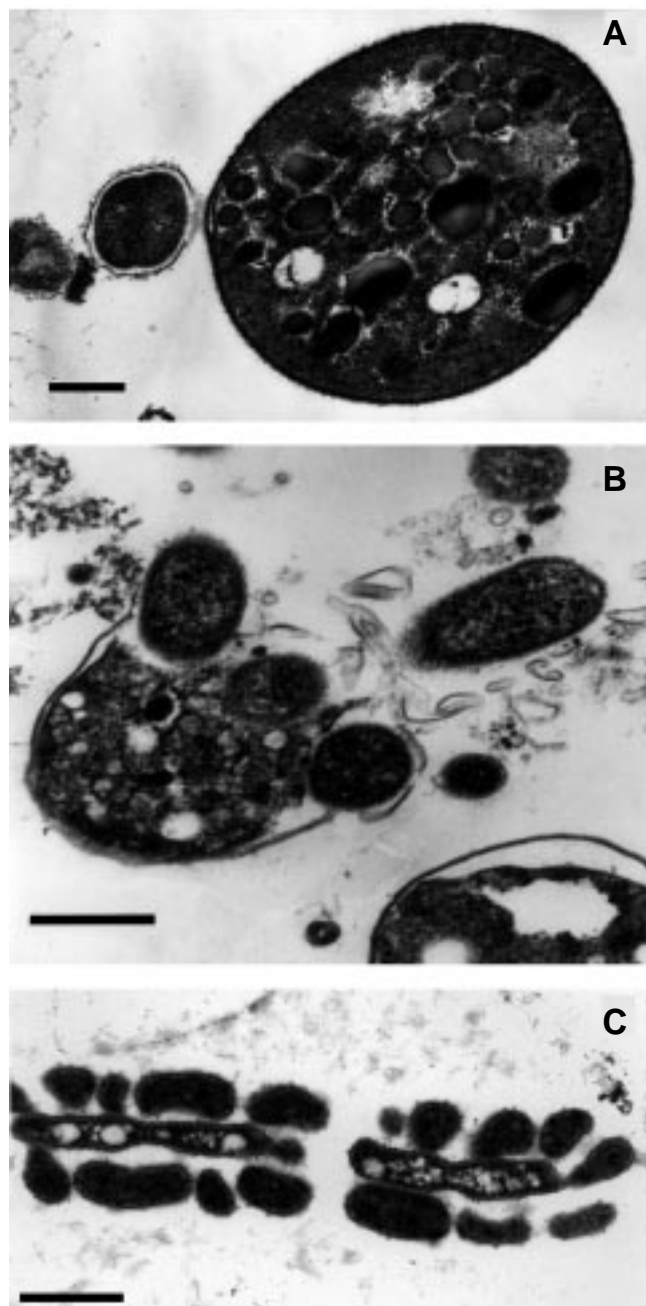


Fig. 1 (A) *Vampirococcus* attached to cell wall of *Chromatium* spp. Bar = 1 µm. (B) Thin section of *Daptobacter* cells inside *Chromatium* spp. Bar = 1 µm. (C) Transmission electron micrograph of a thin section of "*Chlorochromatium aggregatum*" consortia. Bar = 1.2 µm (courtesy of M.A. Martínez, University of Girona, Spain)

51, 56]. It is able to attack a wide range of Gram-negative bacteria, penetrate their cell wall and henceforth generate several progeny swimmers by multiple fission in the periplasmic space of the prey cell.

Several years ago, whilst sampling lake Estanya [35] to study natural samples from the bacterial layers, R. Guerrero

observed a new bacterium unique in its characteristics; it appeared on cell lawns of *Chromatium minus* and then it was isolated from lytic plaques. The infection cycle of this predatory bacterium was determined by transmission electron microscopy by I. Esteve, whereas its morphological and biochemical characteristics were determined by N. Gaju. The bacterium, named by R. Guerrero *Daptobacter*, penetrates both the cell wall and cytoplasmic membrane of its prey. Once inside, it digests the cytoplasm and subsequently divides by binary fission to form two offspring cells. The characteristics of these bacteria and their role in controlling populations of purple phototrophic bacteria have been investigated in karstic lakes [22, 28, 34, 36, 45] (Fig. 1B).

The relationships among microorganisms are not only antagonistic, sometimes stable relationships are mutualistic. Recently two new syntrophic associations between phototrophic and non-phototrophic bacteria were described. Both consortia were observed and collected in the hypolimnion of several lakes [1] (Fig. 1C).

Endo- and ectosymbiosis among bacteria and protoctists

There are many examples of bacterial endosymbionts of different eukaryotes. Endosymbiosis with protoctists are of great interest. Table 1 shows some typical examples of endosymbiosis. Protozoans are often colonized by several bacteria. A recent, yet classical, example was the case of an amoeba-bacteria symbiosis which occurred spontaneously in 1966 when a strain of *Amoeba proteus* became infected with many (60,000–150,000 bacteria per amoeba) rod-shaped Gram-negative bacteria. At first the bacteria were harmful to their hosts. With time, however, not only did the bacteria become less virulent, but they also became necessary to their amoeba host, which lost viability if deprived of their endosymbionts [40].

A new microbial consortium was discovered by Finlay et al. [26]: the partners are the ciliated protozoon *Trimyema* sp. and a single species of methanogen. The consortium has been maintained in culture for more than four years. Each ciliate contains up to 300 symbiotic bacteria which are irregularly disc-shaped and distributed throughout the host's cytoplasm. The symbionts belong to a new species of archaeobacterium which is a close relative of the free-living methanogen *Methanocorpusculum parvum*.

Cyanophora paradoxa represents the most extensively and best investigated species within the Glaucocististophiceae. Note that two strains of *C. paradoxa* have been found with different cyanoplasts which conserve cyanobacterial plasma membranes and are different from the chloroplast envelope membranes of red or green algae [2].

Geosiphon pyriforme, a diphyous fungus, has as a facultative endocytobiont which is a hormogonial cyanobacterium related to *Nostoc punctiforme* [53].

Table 1. Symbioses among organisms. Some examples

Host	Symbiont	Reference
1. Symbioses among bacteria^a		
Different Gram-negative bacteria	<i>Bdellovibrio</i>	57
<i>Chlorella</i>	<i>Vampirovibrio</i>	31
<i>Chromatium</i>	<i>Vampirococcus</i>	23
Different purple bacteria	<i>Daptobacter</i>	36
<i>Thiothrix</i>	<i>Daptobacter</i> -like	45
<i>Micrococcus</i>	<i>Ensifer</i>	6
<i>Pseudomonas</i>	<i>Micavibrio</i>	43
<i>Pelochromatium roseum</i> ^b	ovoid to rod-shaped green bacteria	1
2. Cyanobacterial symbioses		
<i>Geosiphon pyriforme</i>	<i>Nostoc</i>	53
<i>Cyanophora paradoxa</i>	Cyanoplasts	12
3. Prokaryotic symbionts of <i>Amoeba</i> and flagellates		
<i>Myxotricha paradoxa</i>	ectosymbionts	12
<i>Amoeba</i>	symbiosome	50
4. Prokaryotic symbionts of ciliates		
<i>Paramecium octaurelia</i>	<i>Lyticum flagellatum</i>	44
<i>Metopus striatus</i>	<i>Methanobacterium formicicum</i>	59
<i>Euplotes aediculatus</i>	<i>Polynucleobacter necessarius</i>	39
5. Prokaryotic symbionts of animals		
<i>Calyptogenia magnifica</i>	methylophilic bacteria	60
<i>Lucinoma aequizonata</i>	sulfur oxidizers	17
<i>Solemya reidi</i>	idem	14
<i>Riftia pachytila</i>	idem	7
<i>Euprymna scolopes</i>	<i>Vibrio fischeri</i>	52
<i>Polysyneton</i>	<i>Prochloron</i>	18
6. Prokaryotic symbionts of plants		
Wide variety of leguminous	<i>Rhizobium</i>	54
Tropical leguminous trees	<i>Azorhizobium</i>	54
Various tropical leguminous plants	<i>Bradyrhizobium</i>	54

^aPredation and stable microbial associations

^bThe associated microorganisms are called "*Chlorochromatium aggregatum*"

Several different bacterial types have been reported in the cytoplasm of amoebae, mostly enclosed in symbiosomes; they are found single or in groups. Roth [50] was among the first to confirm by electron microscopy the bacterial nature of previously reported bacteria-like particles in vacuoles of *Amoeba proteus*.

Paramecium tetraurelia has an endosymbiotic bacterium named *Lyticum flagellatum*. These bacteria are straight rods, 0.6–0.8 µm × 2.0–4.0 µm. They resemble bacilli in their general appearance and they bear numerous peritrichous flagella, but are not obviously motile. They are enclosed in vacuoles in the cytoplasm of their hosts [44].

Electron microscopic investigations of sapropelic ciliates (those living in anaerobic sediments rich in decaying plant material) revealed the absence of mitochondria and the presence of microbodies. In *Metopus striatus*, a Gram-positive rod-shaped bacterium was regularly found to be in close association with a microbody consisting of a granular matrix surrounded by a membrane [59].

Endosymbiotic bacteria are also very common in *Euplotes*, a ciliate genus that comprises both freshwater and marine species. All except one of the bacterial symbionts of *Euplotes* are confined to the cytoplasm. It appears that most of the *Euplotes* symbionts cannot grow outside their hosts [55]. So far, only one of the symbionts has been given a binomial name *Polynucleobacter necessarius* [39]. They are slightly curved rods (about 0.3 μm x 2.5–7.5 μm). The symbionts are individually contained in vesicles, to which ribosomes are often attached. If stained with DNA-specific dyes, usually 3 to 9 but in some cases up to 12, intensely stained and regularly spaced dots become visible.

Also *Coleps hirtus*, a ciliate collected from Lake Cisó, when ruptured, released *Chlorella*-like algae which did not appear to be digested and which were not observed either in cultures or in the water column of the lake [24].

Examples of an ectosymbiotic relationship are the spirochaetes on *Myxotricha*, which were found to help their host move by their coordinated undulation whereas the host's flagella functioned only to steer its movement [12]. Different free-living spirochaetae from microbial mats have been studied and their morphologies and structures have also been described. [27, 33, 46].

Prokaryotic symbionts of animals and plants

Besides the many associations found in protists, animals and plants are also frequently colonized by bacteria. A most interesting example is *Prochloron*, which is found in nature as a symbiont of marine invertebrates (ascidians). Electron micrographs of thin sections show that *Prochloron* has an extensive thylakoid membrane system similar to that observed in the chloroplast, which contains chlorophyll *a* and *b*, but does not contain phycobilins. Initially, *Prochloron* was thought to be the type of organism that led, following endosymbiotic events, to the green plant chloroplast [18].

Life at the seeps is possible because clams, mussels, and tube worms that thrive there have established a type of symbiosis that may be unique in the animal kingdom [7, 10, 13, 17, 60]. Only three bivalve species have been investigated for mechanisms by which suitable environments for their bacterial symbionts are maintained: *Calymene magnifica*, *Lucinoma aequizonata*, and *Solemya reidi*.

Calymene magnifica is found at hydrothermal vents. These clams can be frequently observed wedged into cracks in the sea floor where warm water with sulfide is emitted. A large fraction of the body weight of the bivalve is formed by blood containing hemoglobin within erythrocytes for oxygen transport. Since the bacteria in the gill cells live in close proximity to the environment, no elaborate transport mechanisms for any other substances appear to be necessary [60].

Lucinoma aequizonata is collected at a depth close to the interface of a hypoxic basin with overlying oxygen-rich waters,

where local oxygen concentrations are very low and almost no sulfide, either bound as metal sulfide or free, is detectable. The bacteria, however, appear to be sulfur oxidizers because they accumulate elemental sulfur in high concentrations. In addition, the clams have no apparent mechanism to concentrate sulfide. This puzzling situation may be explained by the presence of pockets of sulfidic mud in the proximity of the animal. Thiosulfate rather than sulfide may be an energy source for the bacteria [17].

Solemya reidi is the bivalve that has been best investigated for the uptake of substances from the environment. So far this bivalve has been collected only from areas around sewage outfalls and at the outflow of a paper mill. Similar to the case of *Lucinoma aequizonata*, the sulfide is oxidized to thiosulfate by a sulfide oxidase in the host tissue and then used by the symbionts [14].

Symbioses in Pogonophora ("tubeworms") and Vestimentifera are also very similar. In both cases, the bacteria are housed in a tissue inside the worm's body. The transport mechanisms of these animals have been investigated, especially to discover the way sulfide is carried from the environment into the worm's "trophosome". These animals are unique in that their hemoglobin is able to bind sulfide. It is not yet understood how the hemoglobin is triggered to release the sulfide again in the symbiont-containing organ, but it has been shown that sulfide is released in the presence of symbiotic bacteria [7, 10].

Some marine invertebrates and fish establish mutualistic relationships with luminescent bacteria. Past studies have demonstrated that marine luminous bacteria and *Vibrio fischeri* in particular, are remarkably successful at adapting to a variety of ecological niches. At least the four described species form stable, cooperative associations in specialized organs of marine squids and fishes [19, 30, 41, 52].

Aphids and *Buchnera* also have a symbiotic relationship. Aphids are dependent on *Buchnera* for normal growth and reproduction, whereas they supply *Buchnera* with a constant intracellular environment (Fig. 2).

One of the most important mutualistic relationships between microorganisms and plants involves the invasion of the roots of suitable host plants by nitrogen-fixing bacteria, resulting in the formation of a nodule within which the bacteria are able to fix atmospheric nitrogen. Until recently, all nodulating and nitrogen-fixing bacteria associated to leguminous plants were placed into a single genus *Rhizobium*. Now two additional genera, *Azorhizobium* and *Bradyrhizobium* are recognized. *Azotobacter* is a unique member of the group which forms stem nodules on a tropical leguminous tree (*Sesbania rostrata*). *Bradyrhizobium* differs from *Rhizobium* by its slow growth in culture, in the location of the *nod* and *nif* genes, and in its host specificity range [54].

In nature, microorganisms are not isolated. On the contrary, they are permanently associated to other organisms by means of either physical or metabolic relations. Little is known, however, about the biotic and abiotic factors which make it

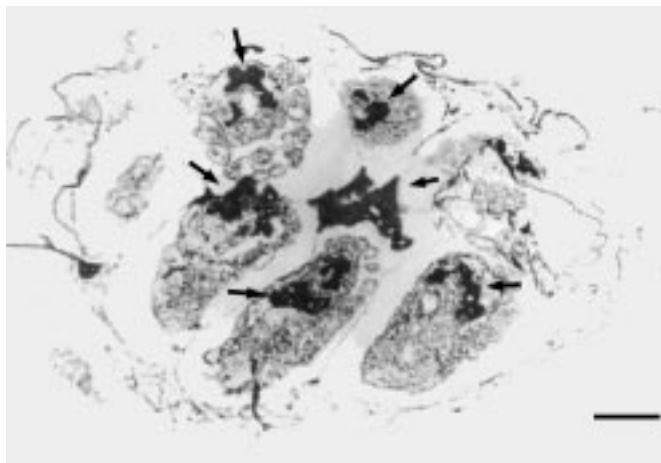


Fig. 2 Phase contrast microscopic image of nonradioactive in situ hybridization of *Rhopalosiphum padi* sections against a fragment of the leucine operon of *Buchnera aphidicola*, the primary endosymbiont of aphids. The image is a 5 mm cross section of the abdominal part of an adult aphid. Arrows indicate mycetocytes corresponding to embryos, while arrowheads point out the maternal mycetocyte. Bar = 100 μ m (courtesy of A. Moya, University of Valencia, Spain)

possible for those associations to become established. Methods used in classical microbiology, such as axenic culture, are a hindrance to the improvement of our current knowledge of symbiotic microorganisms. More work in that field and new methodologies would help to achieve a better knowledge of both microbial diversity and the role microorganisms play in the ecosystem.

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